Fig. 1

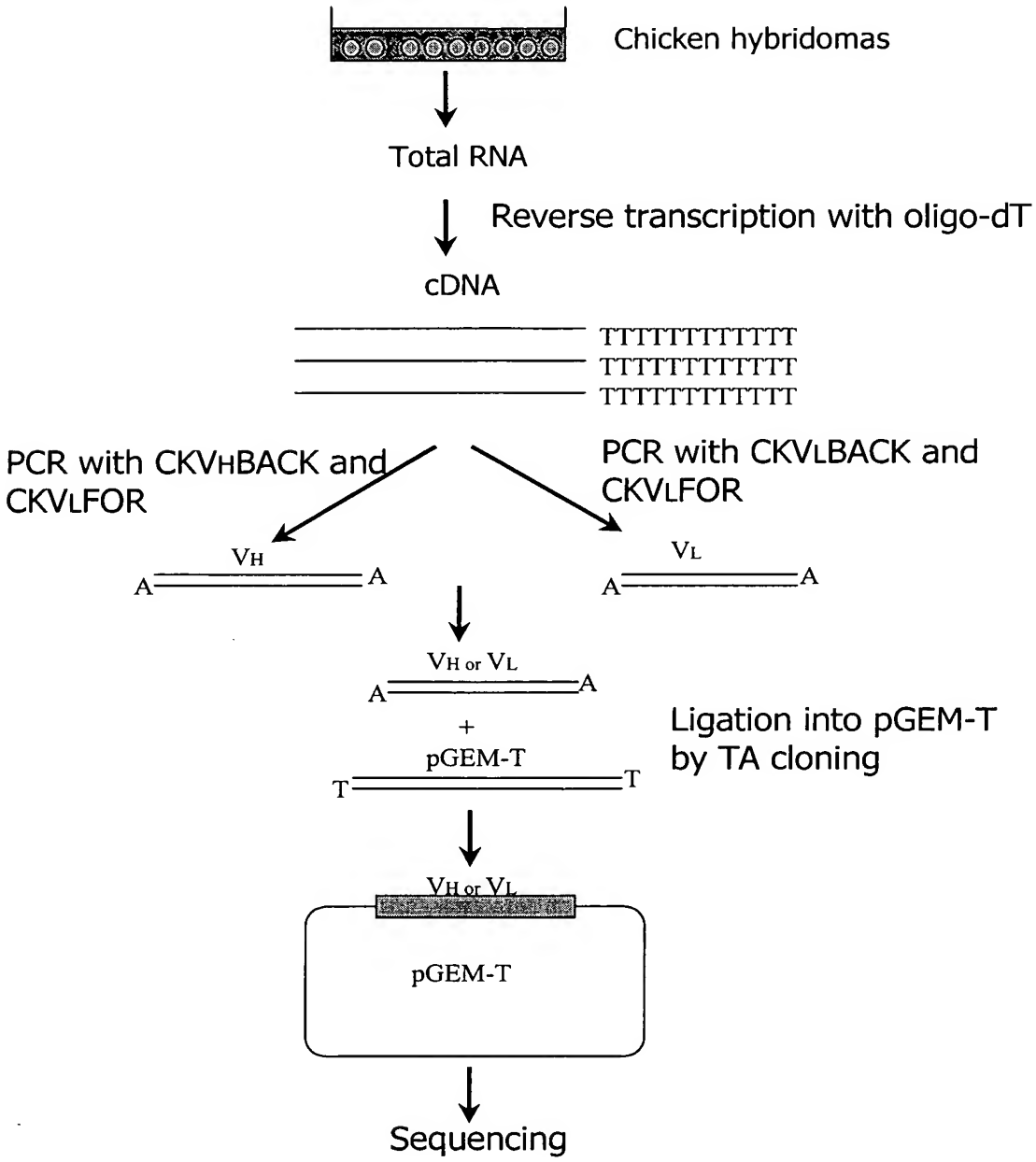

Chicken hybridomas
Total RNA
Reverse transcription with oligo-dT
cDNA
TTTTTTTTTTTT
TTTTTTTTTTTT
TTTTTTTTTTTT
PCR with CKVHBACK and CKVLFOR
PCR with CKVLBACK and CKVLFOR
VH
A
A
VL
A
A
VH or VL
A
A
+
pGEM-T
T
T
Ligation into pGEM-T by TA cloning
VH or VL
pGEM-T
Sequencing

Fig. 2

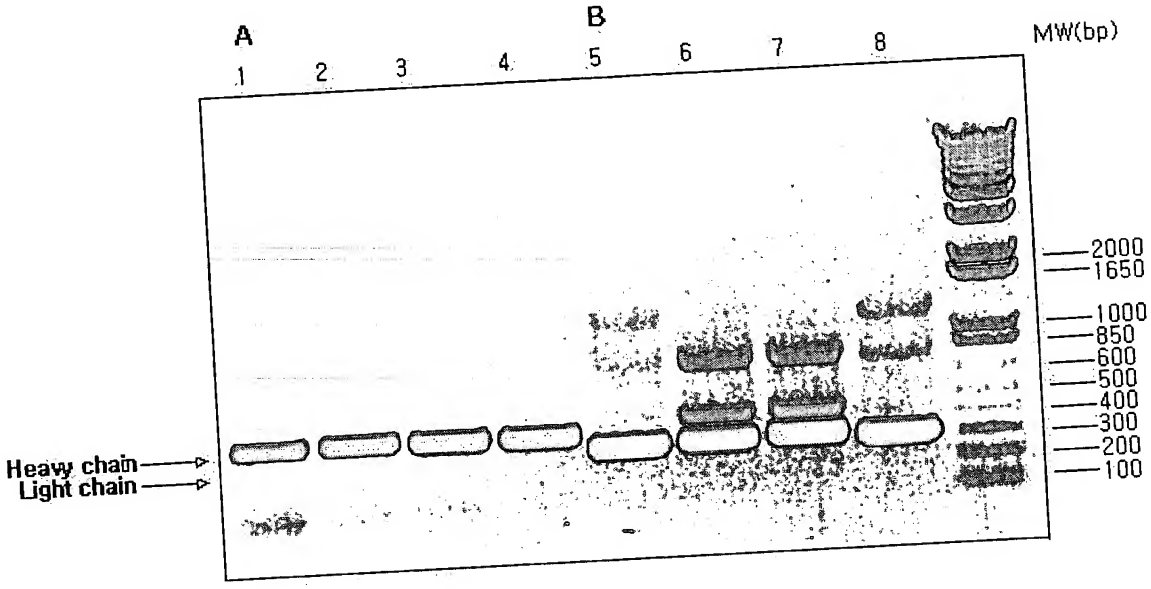

A
B
1
2
3
4
5
6
7
8
MW(bp)
2000
1650
1000
850
600
500
400
300
200
100
Heavy chain
Light chain

Fig. 3a

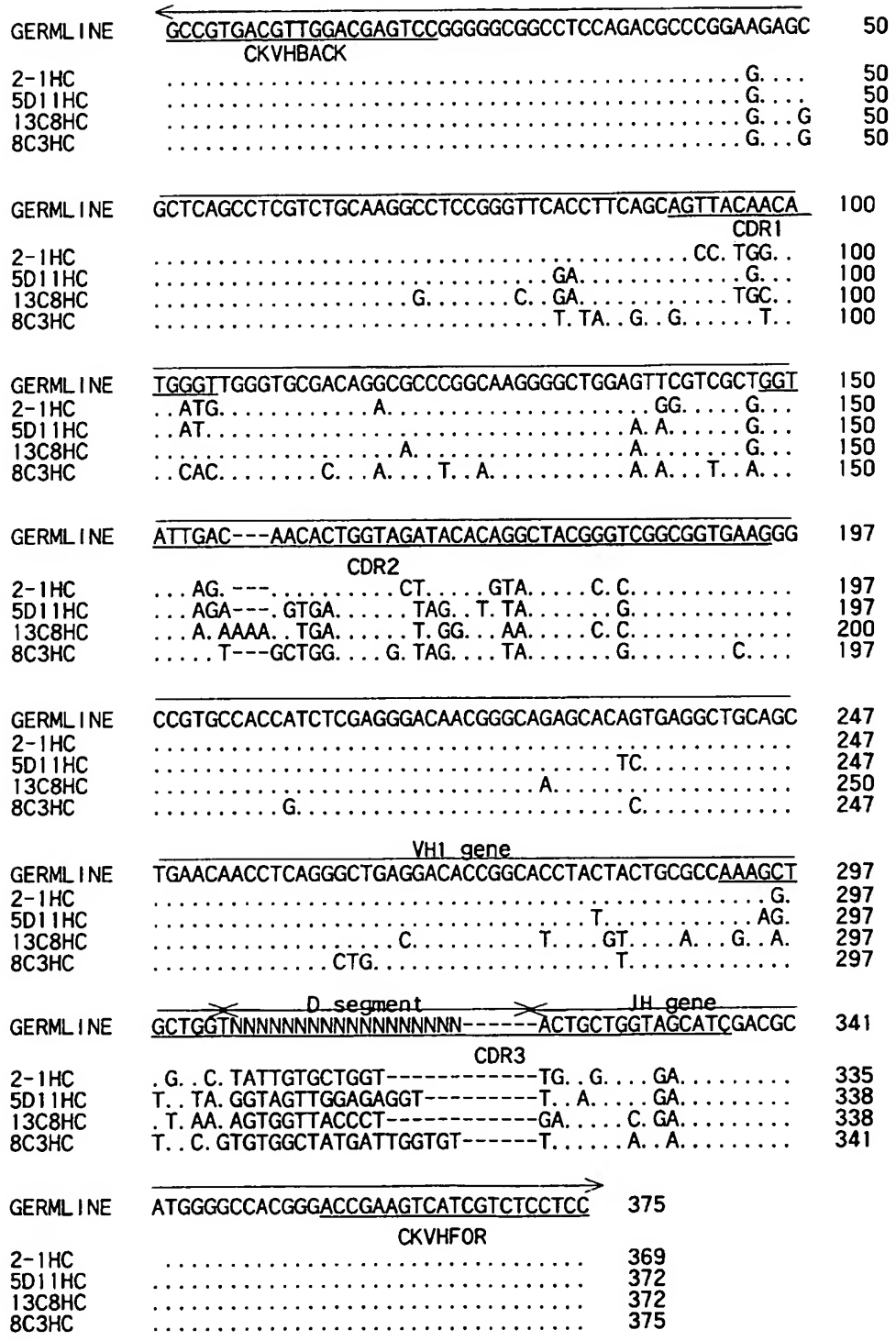

GERMLINE GCCGTGACGTTGGACGAGTCCGGGGGCGGCCTCCAGACGCCCGGAAGAGC 50
CKVHBACK
2-1HC .....G.... 50
5D11HC .....G.... 50
13C8HC .....G...G 50
8C3HC .....G...G 50
GERMLINE GCTCAGCCTCGTCTGCAAGGCCTCCGGGTTCACCTTCAGCAGTTACAACA 100
CDR1
2-1HC .....CC.TGG.. 100
5D11HC .....GA.....G... 100
13C8HC .....G.....C..GA.....TGC.. 100
8C3HC .....T.TA..G..G.....T.. 100
GERMLINE TGGGTTGGGTGCGACAGGCGCCCGGCAAGGGGCTGGAGTTCGTCGCTGGT 150
2-1HC ..ATG.....A.....GG.....G... 150
5D11HC ..AT.....A.A.....G... 150
13C8HC .....A.....A.....G... 150
8C3HC ..CAC.....C...A....T..A.....A.A...T..A... 150
GERMLINE ATTGAC---AACACTGGTAGATACACAGGCTACGGGTCGGCGGTGAAGGG 197
CDR2
2-1HC ...AG.---.....CT.....GTA.....C.C..... 197
5D11HC ...AGA---.GTGA.....TAG..T.TA.....G..... 197
13C8HC ...A.AAAA..TGA.....T.GG...AA.....C.C..... 200
8C3HC .....T---GCTGG....G.TAG....TA.....G.....C.... 197
GERMLINE CCGTGCCACCATCTCGAGGGACAACGGGCAGAGCACAGTGAGGCTGCAGC 247
2-1HC 247
5D11HC .....TC..... 247
13C8HC .....A..... 250
8C3HC .....G.....C..... 247
VH1 gene
GERMLINE TGAACAACCTCAGGGCTGAGGACACCGGCACCTACTACTGCGCCAAAGCT 297
2-1HC .....G. 297
5D11HC .....T.....AG. 297
13C8HC .....C.....T....GT....A...G..A. 297
8C3HC .....CTG.....T..... 297
D segment
JH gene
GERMLINE GCTGGTNNNNNNNNNNNNNNNNN------ACTGCTGGTAGCATCGACGC 341
CDR3
2-1HC .G..C.TATTGTGCTGGT----------TG..G....GA..... 335
5D11HC T..TA.GGTAGTTGGAGAGGT---------T..A.....GA..... 338
13C8HC .T.AA.AGTGGTTACCCT----------GA.....C.GA..... 338
8C3HC T..C.GTGTGGCTATGATTGGTGT------T.....A..A..... 341
GERMLINE ATGGGGCCACGGGACCGAAGTCATCGTCTCCTCC 375
CKVHFOR
2-1HC 369
5D11HC 372
13C8HC 372
8C3HC 375

Fig. 3b

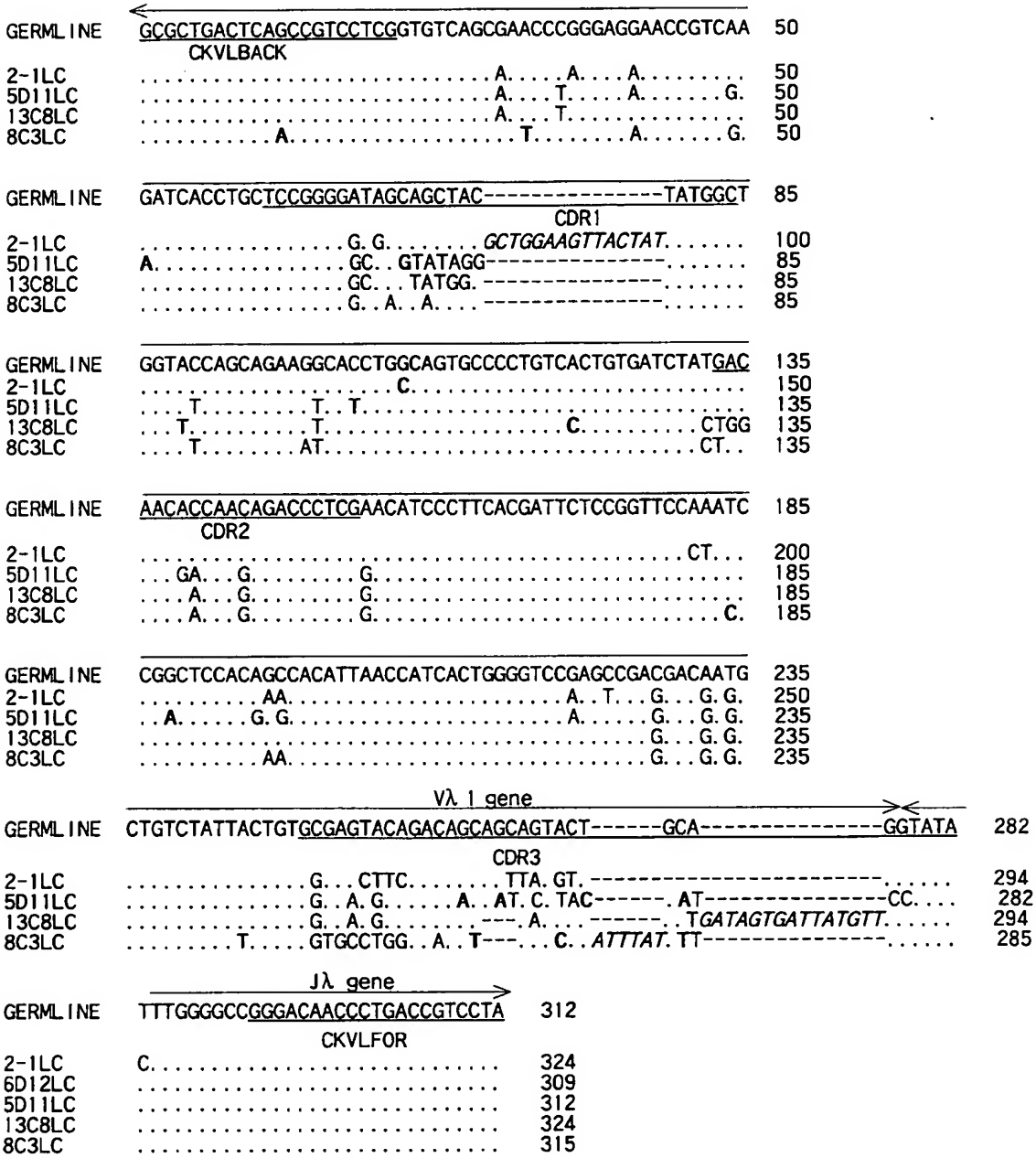

GERMLINE GCGCTGACTCAGCCGTCCTCGGTGTCAGCGAACCCGGGAGGAACCGTCAA 50
CKVLBACK
2-1LC 50
5D11LC 50
13C8LC 50
8C3LC 50
GERMLINE GATCACCTGCTCCGGGGATAGCAGCTAC----------TATGGCT 85
CDR1
2-1LC 100
5D11LC 85
13C8LC 85
8C3LC 85
GERMLINE GGTACCAGCAGAAGGCACCTGGCAGTGCCCCTGTCACTGTGATCTATGAC 135
2-1LC 150
5D11LC 135
13C8LC 135
8C3LC 135
GERMLINE AACACCAACAGACCCTCGAACATCCCTTCACGATTCTCCGGTTCCAAATC 185
CDR2
2-1LC 200
5D11LC 185
13C8LC 185
8C3LC 185
GERMLINE CGGCTCCACAGCCACATTAACCATCACTGGGGTCCGAGCCGACGACAATG 235
2-1LC 250
5D11LC 235
13C8LC 235
8C3LC 235
Vλ 1 gene
GERMLINE CTGTCTATTACTGTGCGAGTACAGACAGCAGCAGTACT------GCA----------GGTATA 282
CDR3
2-1LC 294
5D11LC 282
13C8LC 294
8C3LC 285
Jλ gene
GERMLINE TTTGGGGCCGGGACAACCCTGACCGTCCTA 312
CKVLFOR
2-1LC 324
6D12LC 309
5D11LC 312
13C8LC 324
8C3LC 315

Fig. 3c

```
                                                
```

Fig. 3d

Germline
Primer VLB
CDR1
6D-12-G10
Germline
CDR2
6D-12-G10
---- Vλ1 gene----------/---------- Jλ1 gene ----------
Germline
CDR3
Primer VLF
6D-12-G10

Fig. 4a

```
Germline    AVTLDESGGGLQTPGRALSLVCKASGFTFSSYNMGWVRQAPGKGLEFVAG      50
                                             CDR1
2-1HC       ..............G.............HG.M....T......W...      50
5D11 HC     ..............G...........D....D.I..........Y...      50
8C3 HC      ..............GG..........SIGG.I.H....T......Y...      50
13C8 HC     ..............GG......G..LD....A.................      50

Germline    IDNT-GRYTGYGSAVKGRATISRDNGQSTVRLQLNNLRAEDTGTYYCAKA      99
                 CDR2
2-1 HC      .S..-.T..Y.AP.....................................-      98
5D11 HC     .RSD-.SSIY..A.................L...................S      99
8C3 HC      ..AG-.GS.Y..A..Q....V........L.........L......F....      99
13C8 HC     .KKND.SW.N.AP.........................D...I.V.TRD     100

Germline    AGXXX--XX-----XTAGSIDAWGHGTEVIVSS      124
                 CDR3
2-1 HC      -GGAY--CA-----GCG.D.............       122
5D11 HC     ---SY-----GSWRGST.D.............       123
8C3 HC      SRCGYDWCS-------.DN.............       124
13C8 HC     VNSGY---------PD.AD.............       123
```

Fig. 4b

```
Germline    ALTQPSSVSANPGGTVKITCSGDSSY-----YGWYQQKAPGSAPVTVIY      44
                                  CDR1
2-1 LC      ............E........GG..AGSYY.........A........      49
5D11 LC     ..........L.E..E.....-----GRYR.......SS.........      44
8C3 LC      ..........I..E..E.....GNN.-----.......S.........      44
13C8 LC     ..........L..........G-----SYG...F...S......P...      44

Germline    DNTNRPSNIPSRFSGSKSGSTATLTITGVRADDEAVYYCASTDSSST---  91
              CDR2                                          CDR3
2-1 LC      ................L....N.......QVE.......G.F...YV---  94
5D11 LC     ..DK...D.........D..G.......Q.E.......GNA.NNTY---  91
8C3 LC      Y.NK...D.........P...N.........E.....F.GAWE..PI---  91
13C8 LC     W-NK...D.......................E.......GNA..NTADSD  94

Germline    -AGIFGAGTTLTVL    104
2-1 LC      --..L..........   106
5D11 LC     -DP............   104
8C3 LC      YV.............   105
13C8 LC     YV.............   108
```

Fig. 5

Antibody	FR1	CDR1	FR2	CDR2	FR3	CDR3
5D11	ψV4 and ψV2/7/18/23/24	ψV14	ψV7	ψV7	ψV7/12	V12 and V11
8C3	ψV24	ψV23	ψV7	ψV12	ψV7/10/12, ψV2/8/17, and ψV13	ψV13
13C8	ψV4	ψV14	ψV6 and ψV11	ψV14 and ψV12/13	ψV11	ψV12 and ψV20
2-1	ψV4 and ψV5/8/14	ψV8	ψV8	ψV8	ψV8 and ψV14	ψV14 and ψV3

Fig. 6

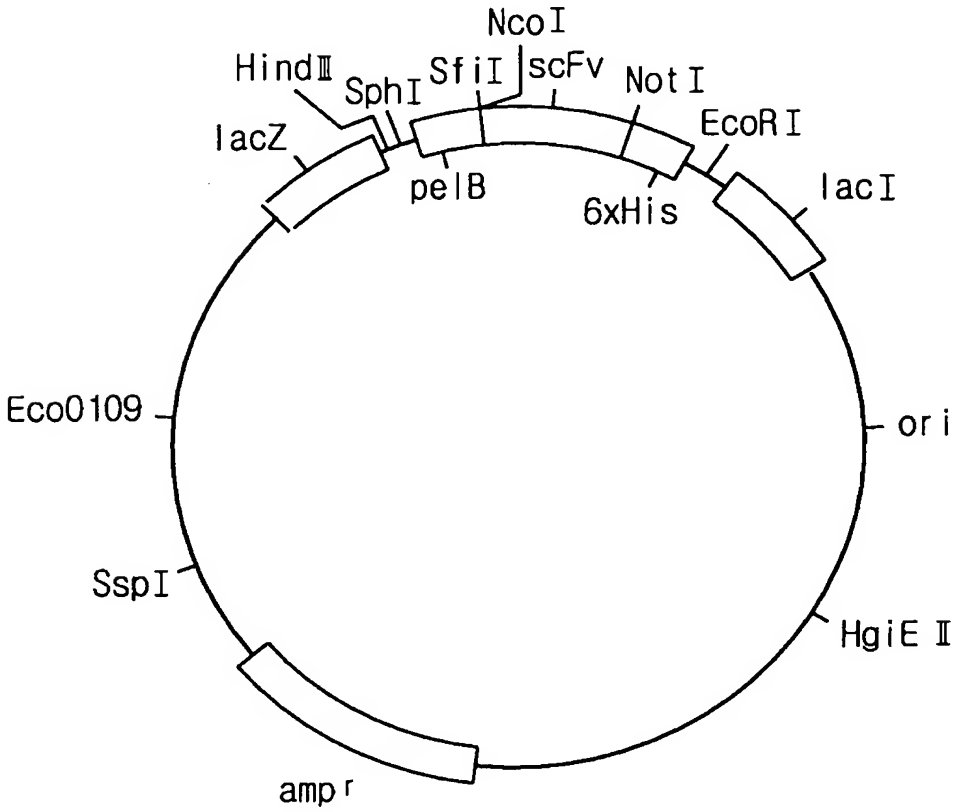
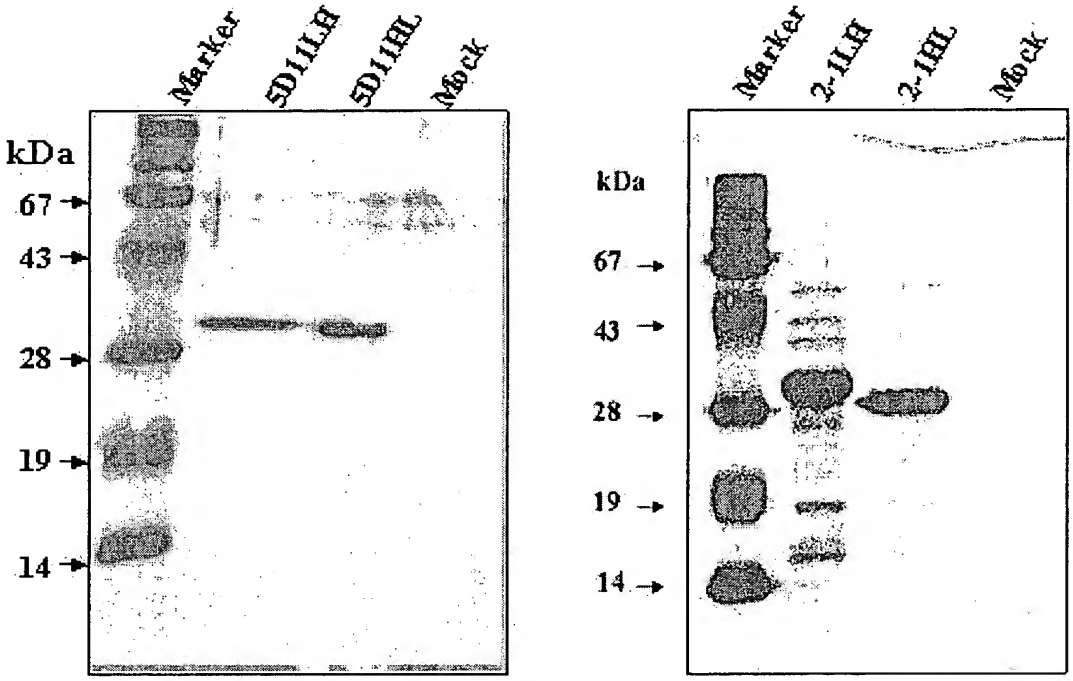

NcoI
HindⅢ
SphI
SfiI
scFv
NotI
EcoRI
lacZ
pelB
6xHis
lacI
Eco0109
ori
SspI
HgiEⅡ
ampr

Fig. 7



Replacement Sheet

Fig. 8

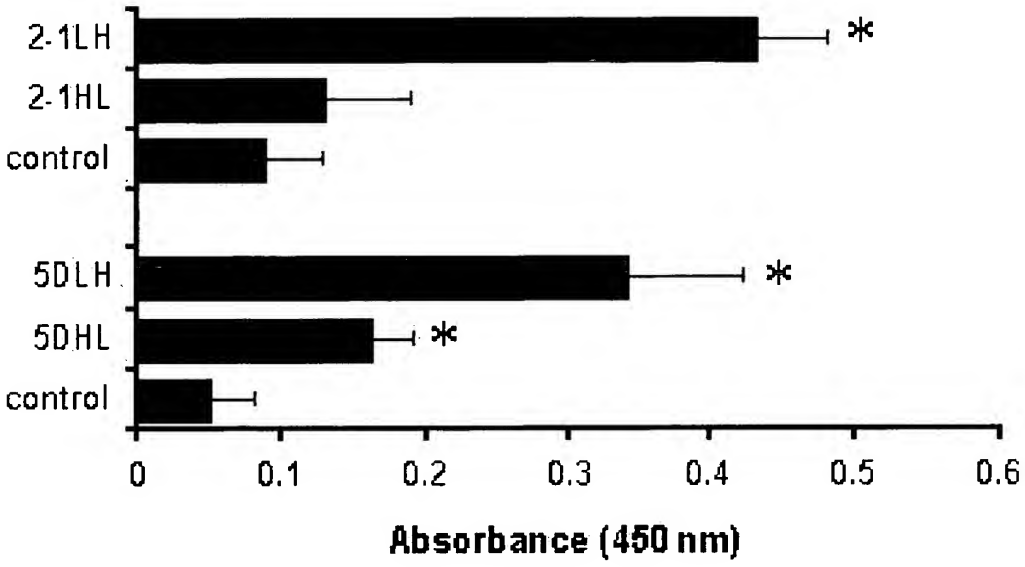
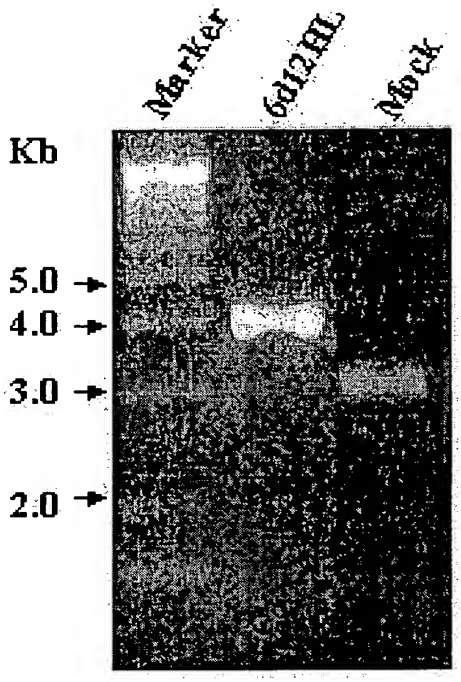
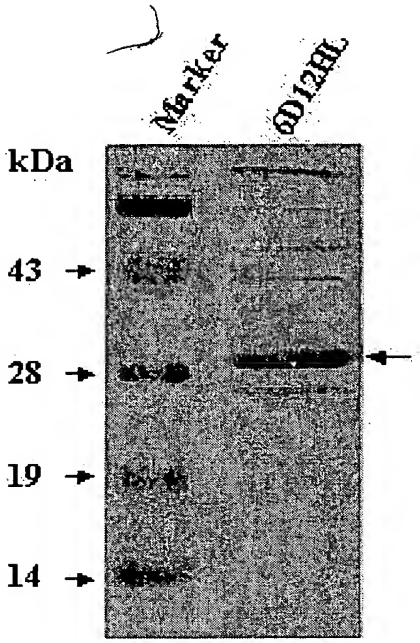

2-1LH
2-1HL
control
5DLH
5DHL
control
*
*
*
0
0.1
0.2
0.3
0.4
0.5
0.6
Absorbance (450 nm)

Fig. 9a



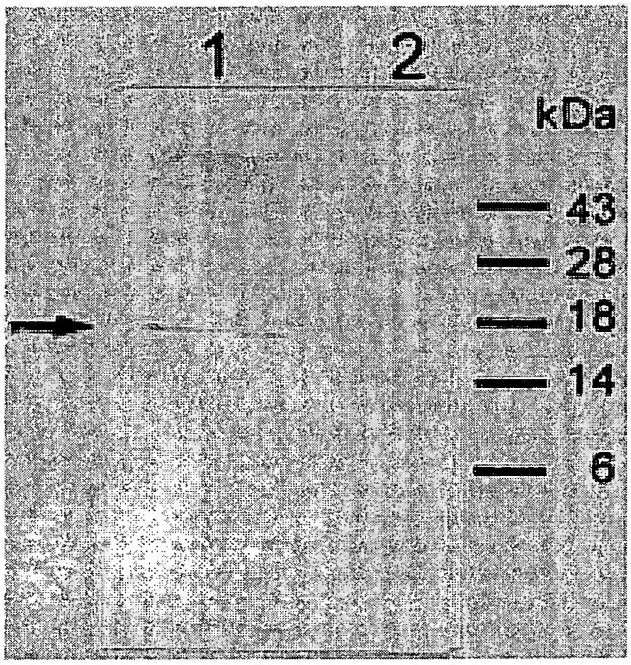
CLEAN COPY

Fig. 9b



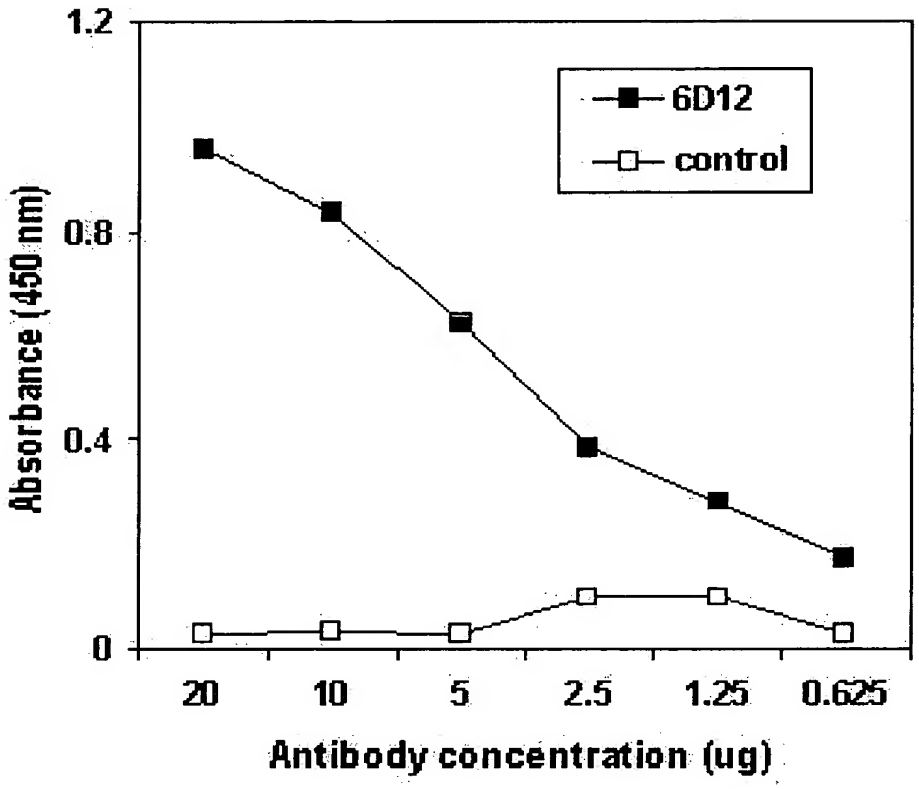
Replacement Sheet

Fig. 10



Replacement Sheet

Fig. 11


1.2
0.8
0.4
0
Absorbance (450 nm)
6D12
control
20
10
5
2.5
1.25
0.625
Antibody concentration (ug)